Journal of Animal Ecology

British Ecological Society

Journal of Animal Ecology 2010, **79**, 1023–1033 doi: 10.1111/j.1365-2656.2010.01718.x

Local exposure shapes spatial patterns in infectivity and community structure of *Daphnia* parasites

Mieke Jansen[1]*, Robby Stoks[1], Ellen Decaestecker[1,2], Anja Coors[1]†, Frank Van De Meutter[1] and Luc De Meester[1]

[1]*Laboratory of Aquatic Ecology and Evolutionary Biology, K.U.Leuven, Ch. Deberiotstraat 32, 3000 Leuven, Belgium; and*
[2]*Aquatic Biology, Science & Technology, I.R.C., K.U.Leuven Campus Kortrijk, E. Sabbelaan 53, 8500 Kortrijk, Belgium*

Summary

1. Spatial patterns in parasite community structure are probably driven by the availability of infectious stages. This is because hosts become infected through picking up infectious stages from their environment. Several studies have, however, reported strong genotype by genotype interactions and parasite-mediated selection in hosts. This leads to the prediction of a parasite by host population interaction with respect to infection rates and intensities, which may also shape spatial patterns in parasite community structure.

2. Using the water flea *Daphnia magna* and its microparasites as a model, we carried out a laboratory experiment to test explicitly to what extent parasite community structure in host populations is determined by the availability of infectious stages in the sediment they are exposed to, and to what extent host population identity and location play a role.

3. We exposed 10 *D. magna* host populations each to sediment of their own habitat and sediment of the other nine habitats, and monitored the parasite community of the resulting experimental populations.

4. Sediment seems to be a strong determinant of parasite infection rates, while there was no overall effect of host population. Sympatric parasite and host population combinations did in most cases not result in significantly different infection rates than allopatric parasite and host combinations. Our results indicate that spore availability could be the key variable determining parasite community structure in natural *Daphnia* populations.

Key-words: infection rates, parasite community structure, spore availability

Introduction

Host–parasite interactions are important drivers of biological diversity. Parasites impact host fitness and population dynamics profoundly (Anderson & May 1986; Mangin, Lipsitch & Ebert 1995; Webster, Gower & Blair 2004) and can have important evolutionary consequences (Ebert 1994; Ebert, Zschokke-Rohringer & Carius 1998; Haag & Ebert 2004). There is substantial spatial variation in parasite occurrence, which is often related to variation in environmental variables either directly or indirectly, through their impact on host population densities and the portfolio of potential host species (Lively 1999; Vogwill, Fenton & Brockhurst 2008). In addition to interspecific differences in parasite preferences and host susceptibility (Maccoll 2009), several studies have reported patterns of host genotype by parasite genotype interactions (Carius, Little & Ebert 2001; Little, Watt & Ebert 2006). Given the presence of both ecological drivers as well as host–parasite co-adaptation (i.e. reciprocal adaptation of the two partner populations to each other) the question emerges to what extent spatial patterns in parasite community structure are influenced by the supply of infectious stages and to what extent they are influenced by local adaptation. As hosts become infected by parasites through picking up infectious stages from their environment, the availability of infectious stages is likely important in determining parasite community structure in host populations. Yet, also infection rate (the percentage of infected host individuals) and intensities (the amount of parasite spores per infected host individual) are likely important in determining the relative abundance of parasites in the community. Several reports describe strong parasite genotype by host genotype interactions (Carius, Little & Ebert 2001; Little, Watt & Ebert 2006) and strong parasite-mediated selection in hosts

*Correspondence author. E-mail: mieke.jansen @bio.kuleuven.be
†Present address: ECT Oekotoxikologie GmbH, Boettgerstr. 2-14, 65439 Flörsheim a.M., Germany.

(Haag & Ebert 2004). We tested the prediction whether this leads to a parasite by host population interaction with respect to infection rates and intensities reflecting local adaptation (Ebert 1994).

Natural populations of the water flea *Daphnia* show a wide variety in levels of parasitism and species composition of their parasite community (Green 1974; Stirnadel & Ebert 1997; Ebert 2008). We here investigated to what extent the level of parasitism of resident *Daphnia* populations is determined by local exposure to parasite infectious stages. We confronted 10 *Daphnia* host populations with natural sediments as a source of parasite infectious stages and characterized the parasite communities that spontaneously developed on the hosts. Natural sediments contain a mixture of infectious stages of various parasites and different genotypes within each parasite species, so that parasite species and their different genotypes compete with each other to infect the host. To disentangle the impact of variation at the 'supply' side (the parasite community of infectious spores) from the impact of variation at the 'recipient' side (genetic variation among host populations), we exposed random sets of clones from the 10 different host populations to sediments of their own habitat (sympatric combination; resident populations) as well as to sediments of the other nine habitats (allopatric combinations). If parasite local adaptation prevails, we expect that parasites in sympatric combinations are more successful in infecting hosts than allopatric parasites. In contrast, if host adaptation prevails, host populations will tend to be more resistant to their sympatric parasite spores than to allopatric parasite species and genotypes. If parasite community structure in the different host populations predominantly reflects the presence and relative abundances of infectious spores in the sediment, this indicates that host population by parasite population interactions are not strong enough to impact detectably parasite community structure. Looking in more detail to the parasite community, we primarily expect local adaptation in the cases were parasites are host genotype specific. It is therefore also useful to contrast the pattern for endoparasites with that observed for epibionts, as the latter have a less intimate interaction with their host.

Materials and methods

THE HOST

The water flea *Daphnia magna* (Straus, 1820) is a planktonic cyclic parthenogenetic crustacean that is a strong competitor in interspecific interactions and occupies a pivotal position in the ecosystem, being an efficient grazer while being very susceptible to fish predation (Lampert 1987). Being filter feeders that may browse on the sediment in the presence of visually hunting predators and under food stress (DeStasio 1993), they may get infected by infectious parasite spores in the sediment (Decaestecker, De Meester & Ebert 2002; Decaestecker *et al*. 2004).

Daphnia is a host for a wide array of parasites, including bacteria, fungi, microsporidia and helminths (Green 1974; Ebert 2008). A distinction can be made between endoparasites, which are parasites that are located within the body of the host, and ectoparasites (epibionts), which are located on the body surface (Ebert 2005). Several studies documented the potential for parasite-mediated selection, local adaptation of the parasite, and genotype by genotype interactions in the *Daphnia* host–parasite system (e.g. Ebert 1994; Carius, Little & Ebert 2001; Duncan, Mitchel & Little 2006; Little, Watt & Ebert 2006; Decaestecker *et al*. 2007; Duncan & Little 2007).

We selected 10 habitats that are known to contain *D. magna* populations (from exploratory field work that involved a set of 32 ponds that were sampled for the presence/absence of *D. magna*). These habitats were also characterized for several environmental variables [presence/absence of fish, land use intensity in the immediate neighbourhood (< 100 m) of the pond, pH, temperature, chlorophyll a, turbidity, conductivity, presence/absence of macrophytes and a pilot screening for the presence of parasites]. We chose five populations in the Leuven region (central Belgium) and five populations in the coastal region of Belgium. Distances between ponds within a region range from 1 to 50 km, while the average distance between both regions is 150 km [co-ordinates see Coors *et al*. (2009)]. Clonal lineages of *Daphnia* from each pond were established as laboratory cultures prior to the experiment as described in Coors *et al*. (2009). For each pond, several sediment samples (down to a maximum depth of about 0·5 m) were taken at different locations in the pond and pooled to represent as much as possible the natural pond assemblage of *Daphnia* dormant eggs and parasite spores. This pooled sample per pond had a volume of about 10 L. After mixing the sediment, *D. magna* dormant eggs were isolated and hatched in a climate room (20 ± 1 °C, 16 h light/8 h dark photoperiod), with artificial fresh water (ADaM – Aachener Daphnien Medium, Klüttgen *et al*. 1994) as medium. Ten clonal lineages were randomly picked out from each population and used in this study as host clones. These clonal lineages were grown in the laboratory under standardized conditions during two generations to minimize maternal effects. The clonal lineages were cultured as individuals in 100 mL ADaM and were fed $1–2 \times 10^5$ cells *Senedesmus obliquus* mL^{-1}.

SEDIMENTS AND PARASITES

We used sediments from the set of 10 ponds that we used to obtain the host clones. Sediment samples were collected in the same way as those for isolation of the host clones, but during a sampling campaign in autumn 2006. As no sediment samples were left over from the sampling campaign in summer, we had to resample the ponds to obtain new sediment for the exposures. A pilot survey showed that these sediments contain two endoparasites [*Pasteuria ramosa* (Metchnikoff) and *Binucleata daphniae*) and three epibiont species (*Vorticella* sp. (Linneaus, 1767), *Amoebidium parasiticum* (Cienskowski, 1861) and *Brachionus rubens* (Ehrenberg)]. A potentially important methodological limitation of our approach is that we only consider parasites that produce dormant stages. Although most microparasites of *Daphnia* are horizontally transmitted and produce dormant stages that build up a sediment spore bank, not all of them do so (Larsson *et al*. 1998; Ebert 2005). Our results will only reflect patterns for those parasites that build sufficiently dense dormant stage banks. Therefore, we defined the *Daphnia* parasite community as the set of five parasites that were picked up from the sediment in our experiment. Those five parasites are known to infect *D. magna*, but some of them (the epibionts) are able to also infect other host organisms. As all habitats were shallow ponds, it is safe to assume that the *Daphnia* in these ponds were indeed effectively exposed to these parasites *in situ*.

Pasteuria ramosa is one of the best studied endoparasites of *D. magna*. It is an obligate endoparasitic bacterium that transmits horizontally through spores that are released from dead infected hosts. These spores build up a parasite spore bank (Ebert *et al.* 1996), which can be used to infect other individuals (Decaestecker *et al.* 2004). Infection of the host by *P. ramosa* leads to castration and gigantism (Ebert *et al.* 2004).

Binucleata daphniae, a microsporidian species, is a parasite of *D. magna* with a moderate virulence. Being a strictly intracellular protist, infections are transmitted horizontally. *Binucleata daphniae* infects the cuticular hypodermis. Despite the importance of this tissue for the host, hosts continue to grow and moult, but life-time fecundity is reduced (Refardt *et al.* 2008).

Vorticella sp. belong to the ciliate family of the Vorticellidae. It is a common epibiont of *Daphnia* (Green 1974). It does not cause much harm to the host, but may reduce its fitness by direct competition for food if they are abundant (Havens 1993). We were not able to identify *Vorticella* individuals to the species level.

Amoebidium parasiticum is a fungus of the Trichomycetes. Tufted colonies are generally formed by tubular vegetative filaments that cleave on the surface of the cladoceran host. *Amoebidium parasiticum* lives on the carapax, antennae and filter system of its host; it is not specific and infects different hosts without preference (Green 1974).

Brachionus rubens is the most widespread and common rotifer that lives epibiontic on cladocerans. It adheres to the carapax of its host by a secretion released by the pedal glands (Green 1974).

EXPERIMENTAL SET-UP

The experiment involved a fully crossed design in which 10 clones per population were exposed to all 10 sediments. This resulted in 10 clones × 10 populations × 10 sediments = 1000 experimental units. Note that the 10 different clones from each population were used as replicates in our analyses, and represent a random sample of the studied populations. There are no replicate observations at the clonal level.

Plastic vessels of 50 mL were filled with 15 mL sediment and 30 mL ADaM and well mixed. The sediment was allowed to settle 1 h before the experiment was initiated by inoculating eight second-clutch juveniles, aged between 24 and 48 h, of one particular host clone. All inoculated individuals were offspring of at least the second generation after start-up of the clonal lineage from a dormant egg. Of each clone, 10 groups of each eight juveniles were inoculated; either on their own sediment or on one of the nine other sediments. During 6 days, the sediment and ADaM medium were daily stirred along a horizontal axis to promote the contact between host and parasite spores in the sediment. By using small vessels and daily stirring we ensured that all *Daphnia* clones, irrespective of differences in phototactic behaviour or other traits, were equally likely to come into contact with parasite spores. *Daphnia* were fed 166×10^3 cells *S. obliquus* mL^{-1} every day. After 6 days of exposure, the *Daphnia* were transferred to 100 mL fresh ADaM vials without sediment. Previous experiments have indicated that an exposure time of 6 days at low food level is enough for parasite infection (Decaestecker *et al.* 2004). Food level was increased from 166×10^3 cells *S. obliquus* mL^{-1} on day 6 to 200×10^3 cells *S. obliquus* mL^{-1} from day 7 onwards. Every other day, the vials were cleaned, neonates were removed and medium refreshed. The experiment ended when animals were 21 days old. This period gives the parasites enough time to grow within the body of the host so that their presence can be visually scored (Ebert 2005).

After 21 days, we scored infection rates for all parasites and spore load for *Pasteuria* infections, both expressed per vial. Infection rates of each parasite were scored as the percentage of living *Daphnia* individuals within a given vial that were infected based on visual inspection using a stereomicroscope as well as a microscope at 400× magnification. For *P. ramosa*, we also quantified spore load for all vials with at least one *Daphnia* infected with mature spores as the average number of mature *P. ramosa* spores per infected *Daphnia* (infection intensity). This was done by squashing individual infected *Daphnia* in 300 μL distilled water and counting mature *P. ramosa* spores with a Bürker counter (Bürker Marienfield, Germany) at 400× magnification (phase-contrast). When none of the exposed animals in a given vial were infected with mature spores, the experimental unit was not included in the analysis for spore load. Non-infected animals in a jar with infected ones were also not considered when calculating spore loads. We did not score spore load in the other parasites.

STATISTICS

We analysed the two response variables separately using general models in PROC MIXED of SAS 9.1 (SAS Institute Inc., Cary, NC, USA). The analyses for parasite infection rate were split per parasite species (for spore load only one parasite species was tested). In the model, we included host population, host population nested in host region, sediment region and sediment nested in sediment region as main factors. Clone identity nested in host population was added as a random variable to take into account that each clone was exposed to the 10 different sediments. We simplified the model by removing interactions that were not significant at the 0·10 level. We arcsin transformed parasite infection rates and log transformed spore load to meet the normality assumption. Because not all clones could be perfectly synchronized, the starting day of the different experimental units varied over a period of 7 days. Including this starting day as a covariate did not impact our results, so we present analyses without this covariate. When analysing infection rate and spore load, we only included sediments in which parasite spores were found to be present. This was a subset of the 10 sediments for the ectoparasite *B. rubens* ($n = 2$ sediments) and the endoparasites *P. ramosa* ($n = 5$) and *B. daphniae* ($n = 2$), whereas for the other parasites all sediments could be incorporated in the analyses. As for *B. rubens* and *B. daphniae* only two sediments were included, we could not test for sediment region and this factor was not included in the model. To correct for the amount of spores present in the sediments, we included infection rate as a covariate in the GLM analyses for spore load.

To explicitly test for local adaptation, we performed a series of contrasts per parasite population. Following Kawecki & Ebert (2004), we regarded the 'local vs. foreign' criterion as diagnostic for the pattern of local adaptation. Under local adaptation it is expected that in a given 'habitat' the local population shows higher fitness than populations from other 'habitats'. To assess local adaptation in parasite populations, this translates into the criterion that for a given *Daphnia* population (the 'habitat' for the parasites), the local parasites (own sediment) should show higher infection rates and spore loads than foreign parasites (other sediments). Therefore, to study parasite local adaptation in more detail, we defined one linear contrast per host population comparing the local sediment with the other sediments per parasite population. Differences in number of spores among sediments may mask any pattern of local adaptation as measured by infection rates. For example, if the local sediment would contain many more spores than the foreign sediments, we would, without correction, find higher infection rates by the local parasites (sediment) even in the absence of local adaptation. Therefore, when

analysing infection rates, we corrected for the amount of spores present in the sediment by dividing the infection rate of each vial by the average infection rate across all *Daphnia* individuals exposed to that sediment (so also including *Daphnia* from other ponds). Note that this correction is not based on a direct count of spores, but indirectly done by dividing the infection rate of each vial by the average infection rate across all *Daphnia* individuals exposed to that sediment. Given that we exposed each sediment to 10 populations that can be considered a random sample of regional host populations, our data in our opinion do allow to standardize dose effects by this procedure. Averaging the impact on 10 populations is probably a very good approximation of the relative abundance of infectious spores in the sediments (actually likely to be better than counts, as counts do not provide information on infectivity). Some contrasts were not studied, i.e. for the parasites *B. rubens* and *B. daphniae*, because those parasites were only present in two sediments. Spore load data were corrected for infection rate, in compliance with the GLM analyses.

From the viewpoint of the *Daphnia* populations, the 'local vs. foreign' criterion for local adaptation suggested by Kawecki & Ebert (2004) translates into the prediction that when confronted with a given set of spores from one parasite population (i.e. one sediment sample), the local *Daphnia* population should show lower infection rates and spore loads than foreign *Daphnia* populations. Therefore, we defined a set of orthogonal linear contrasts comparing subgroups of host populations per sediment. A first contrast tested for differences in infection rate or spore load between sympatric (*Daphnia* host population exposed to the parasites of the same pond) and allopatric (local parasites exposed to hosts of the other nine ponds) parasite–host combinations. A second contrast tested within the latter group for differences in infection rate or spore load between naïve (*Daphnia* host populations from a pond of which the sediment does not contain detectable parasite spores of the species considered) and experienced (*Daphnia* host populations from a habitat of which the sediment contains parasite spores of the species considered) populations. The second contrast could not be considered when examining the ectoparasites *Vorticella* sp. and *A. parasiticum* because no naïve populations were present in the data set.

To explore parasite community structure as observed in our experiment in relation to parasite exposure (sediment), host source habitat and environmental variables of the source habitats, we carried out a multivariate analysis on infection rate data using CANOCO (Canoco for Windows version 4.5, Biometris-Plant research International, Wageningen, The Netherlands). We applied a linear canonical method redundancy analysis (RDA), because an initial detrended correspondence analysis (DCA) (Hill & Gauch 1980) suggested that parasite infection rates showed mainly linear responses. To assess whether densities of hosts are related to parasite community composition, we used the density of dormant stages of *D. magna* (expressed as # ephippia g^{-1} sediment) as a proxy for densities of active populations and included this variable in the RDA. When the variable egg count was used to correct for *Daphnia* densities in the RDA, we did not include factors like host population or sediment, as there is a one-to-one relationship between egg count and sediment/host population. In this analysis, the host population–sediment combination was rescaled to 'own' (if the population was exposed to the own sediment) or 'other' (if the host population was exposed to another sediment).

To assess the impact of spatial patterns, we introduced distances for each combination of parasite exposure and host source into the models. We assessed the (correlative) contribution of each individual factor to the parasite community patterns by correcting for all other factors in the model and determined significance of the contributions through 999 Monte Carlo permutations (Legendre & Legendre 1998).

Results

PARASITE INFECTION RATE

All analysed sediments contained dormant stages of at least two of the five scored parasites (endoparasites and/or epibionts). Only six sediments contained one or both of the two endoparasites *Pasteuria ramosa* and *Binucleata daphniae*. In total, three epibionts were observed: *Vorticella* sp., *Brachionus rubens* and *Amoebidium parasiticum*. Epibionts were more common than endoparasites, with the generalist species *Vorticella* sp. and *A. parasiticum* being found in all sediment samples (Fig. 1). *Brachionus* and the endoparasites were more restricted to specific ponds, with *B. rubens* and *B. daphniae* only being detected from two sediments and *P. ramosa* being found in five sediments.

The general linear models identified a strong sediment effect on the infection rate of all parasites (Table 1, Fig. 1). For *P. ramosa*, sediments from Knokke In and Knokke Nat resulted in the highest infection rates (Fig. 2 right panel). For *A. parasiticum*, this was the sediment from Knokke In (Fig. S1 right panel, Supporting Information), and for *Vorticella* sp. the sediment from Knokke Nat (Fig. S2 right panel). Only two sediments induced infections with *B. daphniae*, with OM 1 resulting in higher infection rates than Blankaart (Fig. 3 right panel). Similarly, two sediments induced infections of *B. rubens*, with Moorsel resulting in much higher infection rates than Tersaart (Fig. S3 right panel). Effects of sediment region were found for all three parasites for which

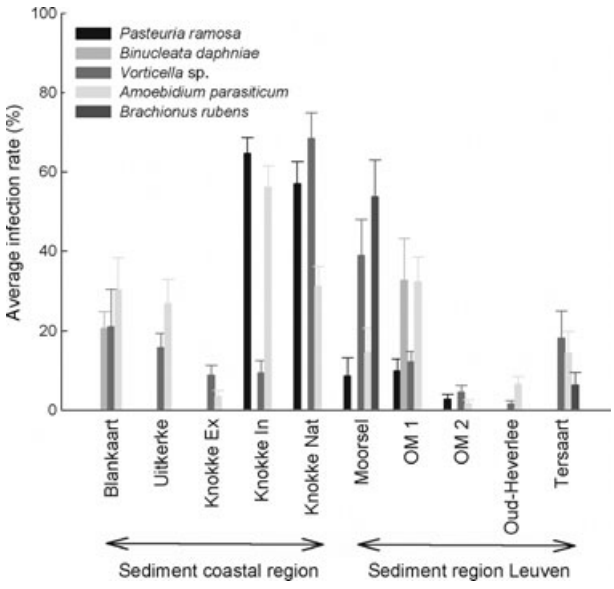

Fig. 1. Average (over clones and populations) infection rate of two endoparasites (*Pasteuria ramosa* and *Binucleata daphniae*) and three epibionts (*Vorticella* sp, *Amoebidum parasiticum* and *Brachionus rubens*) in *Daphnia magna* exposed to 10 different sediments. Mean values are given with 1 SE.

Table 1. Results of general linear models testing for effects of host region (H_region), population nested in host region, sediment region (S_region) and sediment nested in sediment region on parasite infection rate for the five parasite species detected in this study. For *Brachionus rubens* and *Binucleata daphniae*, the full model was not applied due to the presence of these parasites in only two sediments. For those two parasites, we could not include sediment region. The significance of the *F*-values is indicated as follows: *$P < 0{\cdot}05$, **$P < 0{\cdot}01$ and ***$P < 0{\cdot}001$

	Pasteuria ramosa		*Amoebidium parasiticum*		*Vorticella* sp.	
Part A	d.f.	*F*-value	d.f.	*F*-value	d.f.	*F*-value
Population (H_Region)	8;318	5·67***	8;85	1·06	8;847	1·38
H_region	1;318	2·09	1;84·9	6·35*	1;847	0·51
Sediment (S_Region)	3;318	2·67*	8;847	28·64***	8;847	33·83***
S_Region	1;318	464·05***	1;847	88·67***	1;847	36·23***
Population (H_Region) × S_Region	8;306	0·76	8;830	1·62*	8;830	0·92

	Binucleata daphniae		*Brachionus rubens*	
Part B	d.f.	*F*-value	d.f.	*F*-value
Population (H_Region)	8;85	2·85**	8;79	1·76
H_region	1;85	89·95***	1;79	0·11
Sediment	1;85	8·38**	1;79	55·16***
Sediment × H_Region	1;85	28·26***	1;79	1·80
Sediment × Population (H_Region)	8;85	5·63***	8;79	0·78

we could test this effect: *P. ramosa*, *A. parasiticum* and *Vorticella* sp. (Table 1 part A). For these parasites, the average infection rates when exposed to sediments from the coastal region were higher than the average infection rates obtained after exposure to sediments from the Leuven region (Figs 2, S1 and S2 right panels). Contrast analyses for local adaptation in the same three parasites (*P. ramosa*, *A. parasiticum* and *Vorticella* sp.) did not reveal any significant difference in infection rate between exposures of host populations to their own sediment or to the other sediments (all $P > 0{\cdot}05$, Figs 2, S1 and S2 left panels, Table 1 upper panel).

A host population effect on infection rate was present in *P. ramosa* and *B. daphniae* (including an interaction with sediment) but not in the other three parasites (Table 1). Infection rates for both *A. parasiticum* and *B. daphniae* are on average higher in host populations derived from the Leuven region (significant effect of host region, Table 1, Figs 3 and S1). Focusing on the differences in infection rate between the different host populations exposed to the same sediment (host local adaptation), we could not detect differences between sympatric vs. allopatric and naïve vs. resident populations (all $P > 0{\cdot}05$), except for *B. daphniae*. Contrast analyses for the latter parasite revealed for Blankaart sediment a lower infection rate in the resident Blankaart *Daphnia* population compared to all other populations ($F_{1,85} = 4{\cdot}92$; $P = 0{\cdot}029$), but no difference between naïve and experienced populations ($P > 0{\cdot}05$, Fig. 3 left panels). For OM 1 sediment, *B. daphniae* infection rates did not differ between the resident and the other populations ($F_{1,85} = 0{\cdot}49$; $P = 0{\cdot}49$), but were lower in the experienced population Blankaart compared to the eight naïve populations ($F_{1,85} = 12{\cdot}95$; $P < 0{\cdot}001$, Fig. 3 left panels).

The results of the multivariate analyses on parasite community structure largely reflected the separate GLM analyses on infection rate. Sediment was the single important factor, explaining 74% of the variation in parasite species composition (constrained RDA, $P < 0{\cdot}01$, Fig. 4). Population had no significant effect on the parasite community (RDA, $P = 0{\cdot}10$, 4% of the variation explained). Differences in parasite community structure among sediments were unrelated to the measured environmental characteristics of the ponds (constrained RDA, $P = 0{\cdot}99$, 4% variation explained). We ran the same statistical model on the subset of five ponds (Knokke In, Knokke Nat, Moorsel, OM 1, OM 2) and three microparasites (*P. ramosa*, *A. parasiticum*, *Vorticella* sp.) that occurred in all five of these ponds, but the results were similar to the overall analysis (sediment: $P < 0{\cdot}01$, 81% variation explained; population: $P = 0{\cdot}38$, 4% variation explained). Introducing ephippium density and distance as covariables into the above RDA models did only weakly affect these results. Ephippium density had only a small effect on the infection patterns both in the model including all sites (ephippium density: $P = 0{\cdot}005$, 5·2% variation explained; distance: $P = 0{\cdot}90$, 0·3% variation explained) and in the model including the five selected sites (ephippium density: $P = 0{\cdot}26$, 5·7% variation explained; distance: $P = 0{\cdot}91$, 0·7% variation explained).

PASTEURIA RAMOSA SPORE LOAD

The highest spore loads were observed in *Daphnia* hosts that were exposed to sediments from Knokke In and Knokke Nat, both situated in the coastal region. In line with this, the

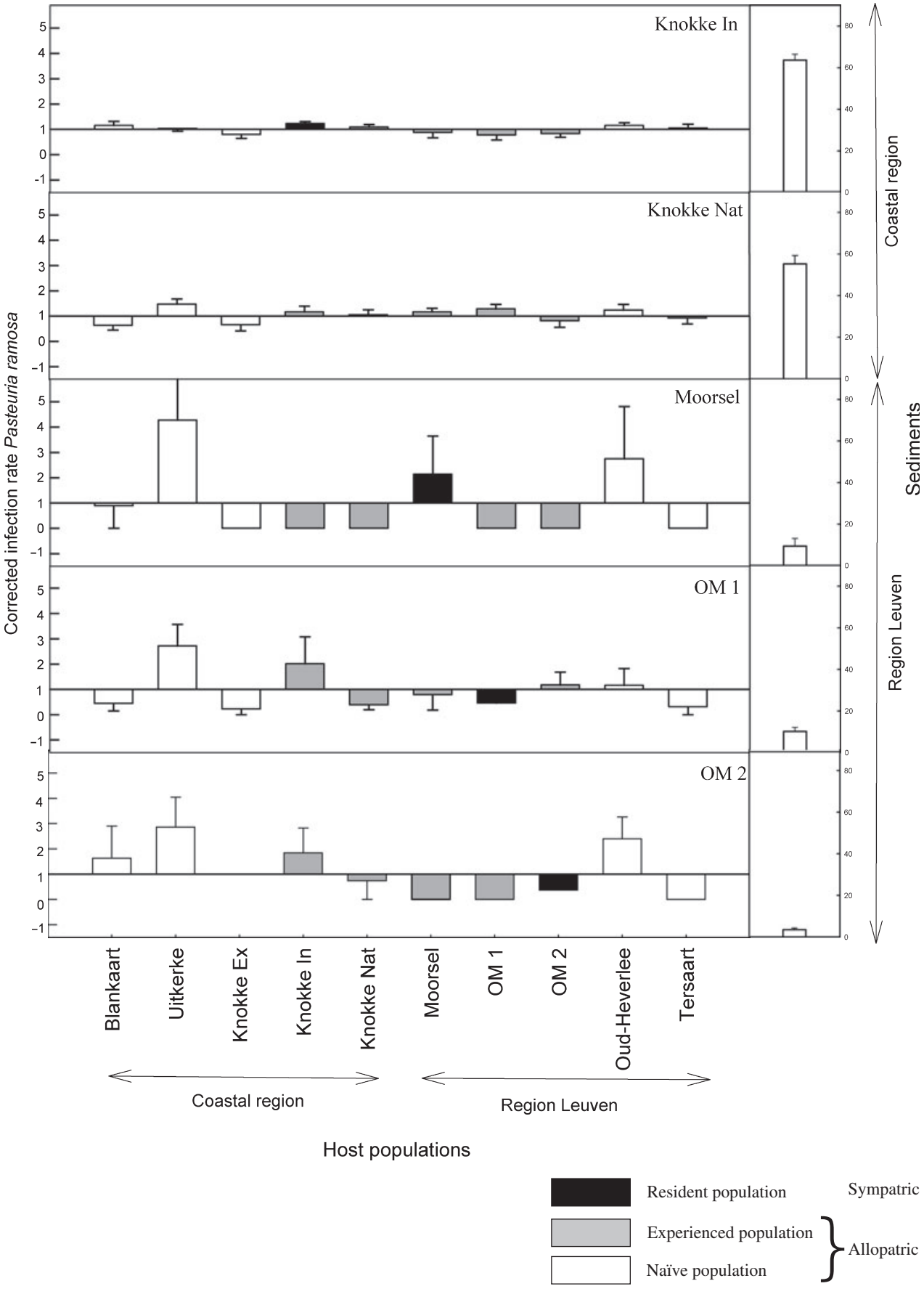
Knokke In
Knokke Nat
Moorsel
OM 1
OM 2
Corrected infection rate *Pasteuria ramosa*
Coastal region
Sediments
Region Leuven
Blankaart
Uitkerke
Knokke Ex
Knokke In
Knokke Nat
Moorsel
OM 1
OM 2
Oud-Heverlee
Tersaart
Coastal region
Region Leuven
Host populations
Resident population
Experienced population
Naïve population
Sympatric
Allopatric

Fig. 2. Average (+1 SE) corrected infection rate of *Pasteuria ramosa* in the 10 *Daphnia* host populations when exposed to the sediments of the five ponds (Knokke In, Knokke Nat, Moorsel, OM 1 and OM 2) containing *P. ramosa* spores (separate panels). Infection rates were corrected by the average infection rate of a given sediment (see Materials and methods); these averages are given for each sediment in the right panel. Note that by doing so the average corrected infection rate is one. Corrected infection rates are therefore shown relative to one, host populations with a larger than average infection rate have a value higher than one, host populations with a lower than average infection rate have a value lower than one.

effect of sediment region was significant ($F_{1,171}$ = 46·93; P < 0·0001); Fig. 5 right panel). Contrast analyses testing for parasite local adaptation indicated that the OM 2 host population suffered higher spore loads when confronted with resident parasites than when confronted with other parasite populations (vertical comparisons in Fig. 5; $F_{1,16}$ = 52·62; P < 0·001). Although there is a tendency for OM 2 *P. ramosa* to show higher spore loads on their local host population than on the other host populations (horizontal comparisons in Fig. 5), this effect is not significant ($F_{1,6}$ = 2·67; P = 0·15). In contrast, two other host populations showed on average lower spore loads when confronted with sympatric than with allopatric parasites (vertical comparisons in Fig. 5, OM 1: $F_{1,13}$ = 5·46; P = 0·036; Moorsel: $F_{1,9}$ = 8·27; P = 0·018). In Knokke In and Knokke Nat, there was no difference in spore load between sympatric and allopatric host–parasite combinations.

There was a significant host population effect on spore load ($F_{8,119}$ = 5·05; P < 0·001). Furthermore, spore loads differed significantly between regions ($F_{1,170}$ = 8·74; P = 0·004), with on average higher spore loads in populations from the coastal region than in those from the Leuven region. Contrast analyses could not detect any difference between resident, naïve and experienced populations (all P > 0·05).

Discussion

Our results indicate that the presence and relative abundance of infectious stages of parasites in the habitat is a very

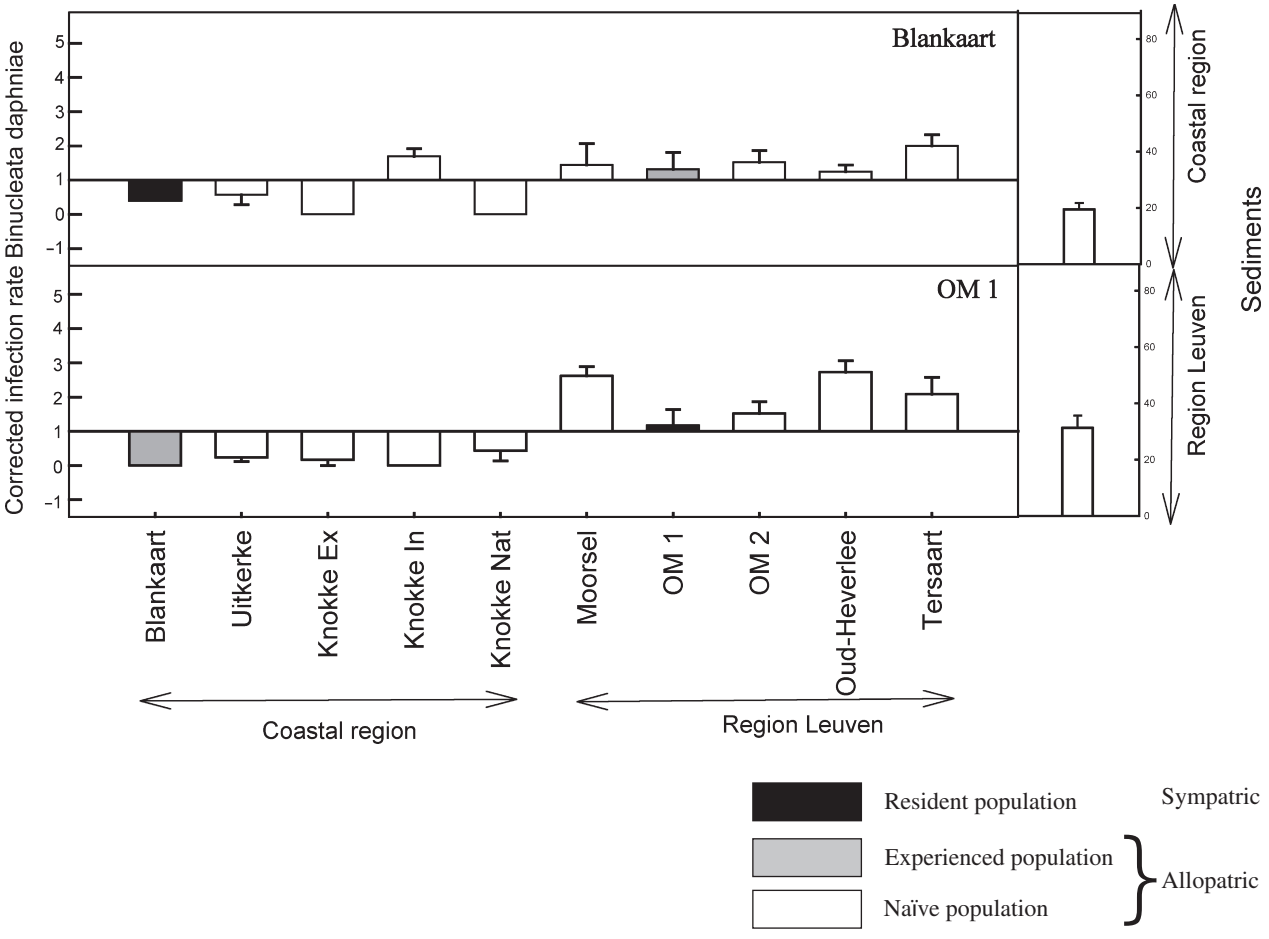

Fig. 3. Average (+1 SE) infection rate of *Binucleata daphniae* in the 10 *Daphnia* host populations when exposed to the two sediments (separate panels) containing *B. daphniae* spores (Blankaart, OM 1). Infection rates were corrected by the average infection rate of a given sediment (see Materials and methods and legend Fig. 2); these averages are given for each sediment in the right panel.

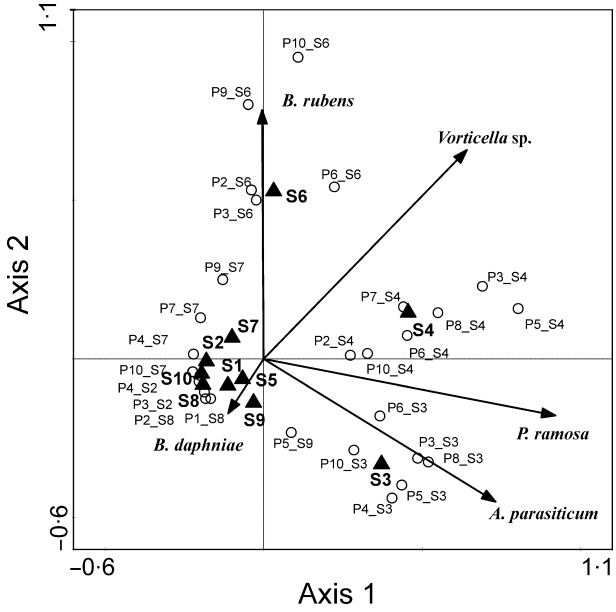

Fig. 4. RDA biplot of *Daphnia* parasite community composition. All sediment–*Daphnia* host population combinations were included in the analysis. Closed triangles indicate the sediment centroids and arrows show the relationship with the presence of specific parasites. Each circle represents a sediment–host population combination (average of the 10 clones for one population) and is coded as P for the population and S for the sediment. Sediment–host population combinations strongly clustered around the axes crossing point; to ensure readability, we only show dots that represent combinations that have a reasonable fit (> 20%) in the ordination space of the biplot. Populations are code as follows: 1 Blankaart, 2 Knokke Ex, 3 Knokke In, 4 Knokke Nat, 5 Uitkerke, 6 Moorsel, 7 Tersaart, 8 Oud-Heverlee, 9 OM 1 and 10 OM 2.

important factor determining taxon composition of the studied parasite community of the host *D. magna*. Several studies have provided convincing evidence of host genotype by parasite genotype interactions and their importance in determining parasite infection dynamics (Lively 1989; Ebert 1994; Manning, Woolhouse & Ndamba 1995; Mopper 1996; Ebert, Zschokke-Rohringer & Carius 1998; Lively & Dybdahl 2000). Our study, however, also reveals that, in the face of the overwhelming variation in community composition that results from differences in relative abundances of infectious stages host population identity had no significant impact on parasite abundance patterns across habitats, and was not the key driver of parasite community composition (only 4% of total variance explained). Our study is unique in the fact that it confronts random samples from various habitats in a given region with random samples of parasite infectious stages of these same habitats in a full factorial design with a focus on parasite community. We did not work with single isolated parasite strains, increasing the ecological realism of our experiment.

One may argue that our result on parasite composition is fully expected because it is obvious that parasites cannot be observed in any host population if they are not present as infectious spores in the sediment. However, if we carry out the RDA analysis including only populations that share the same set of parasites (Knokke In, Knokke Nat, Moorsel, OM 1 and OM 2 for *P. ramosa*, *A. parasiticum*, and *Vorticella* sp.), we obtain a very similar result, with sediment explaining a large part of the total variance in parasite community composition while the effect of host population is not significant. Although we may not have included all *Daphnia* parasites present in the ponds (see Materials and methods; our parasite communities are determined by the species that are picked up from sediments using the methods described), we can conclude that local exposure is a key factor driving spatial patterns in infectivity in the studied community of *Daphnia* parasites.

It is intriguing that despite the proof of strong genotype by genotype interactions for some of the parasites included in this study (e.g. for *Pasteuria ramosa* in Carius, Little & Ebert 2001), this did not translate in strong patterns of parasite by host population interactions reflecting local adaptation at the scale of the current study. One reason that the signal of spatial local adaptation was weak may be that there is an ongoing arms race between hosts and parasites (e.g. in *Daphnia–Pasteuria* interactions, see Decaestecker *et al.* 2007). The resulting temporal dynamics may have produced a lot of variation, possibly to an extent that the signal of spatial genetic variation is strongly reduced. It indeed seems that patterns of temporal adaptation do not necessary translate into patterns of spatial adaptation, and vice versa (Gaba & Ebert 2009; but see Gandon *et al.* 2008). Another reason for the patterns observed by us may be that by maximizing ecological realism and not focusing on a detailed analysis of the responses of single genotypes, we were minimizing the chances of detecting parasite by host population interactions. It was, however, precisely our goal to explore to what extent these genotype by genotype interactions, as typically studied in the laboratory through highly standardized cross-infection experiments, translate into detectable spatial patterns at the parasite community level.

Although our results emphasize that parasite infectious stage supply is much more important than host population identity in determining parasite community composition, we do observe evidence of some degree of local adaptation in our data set. Although infection rates did not reveal local adaptation in those parasite species (*P. ramosa*, *A. parasiticum* and *Vorticella* sp.) for which we could contrast infection rates on sympatric or allopatric host populations, infection rates for *A. parasiticum* and *B. daphniae* were on average higher in host populations from the Leuven than from the coastal region. This is in agreement with predictions from host adaptation scenarios, given that the coastal habitats show higher levels of parasite infectious stages. More direct evidence for local adaptation is observed for the endoparasite *B. daphniae*, for which the host population from Blankaart, one of the two populations that hosted this endoparasite, showed a significantly lower infection rate than the other infected host populations. For the other endoparasite, *P. ramosa*, contrast analyses on spore loads testing for parasite local adaptation indicated significant higher (one case) and lower (two cases) spore loads when

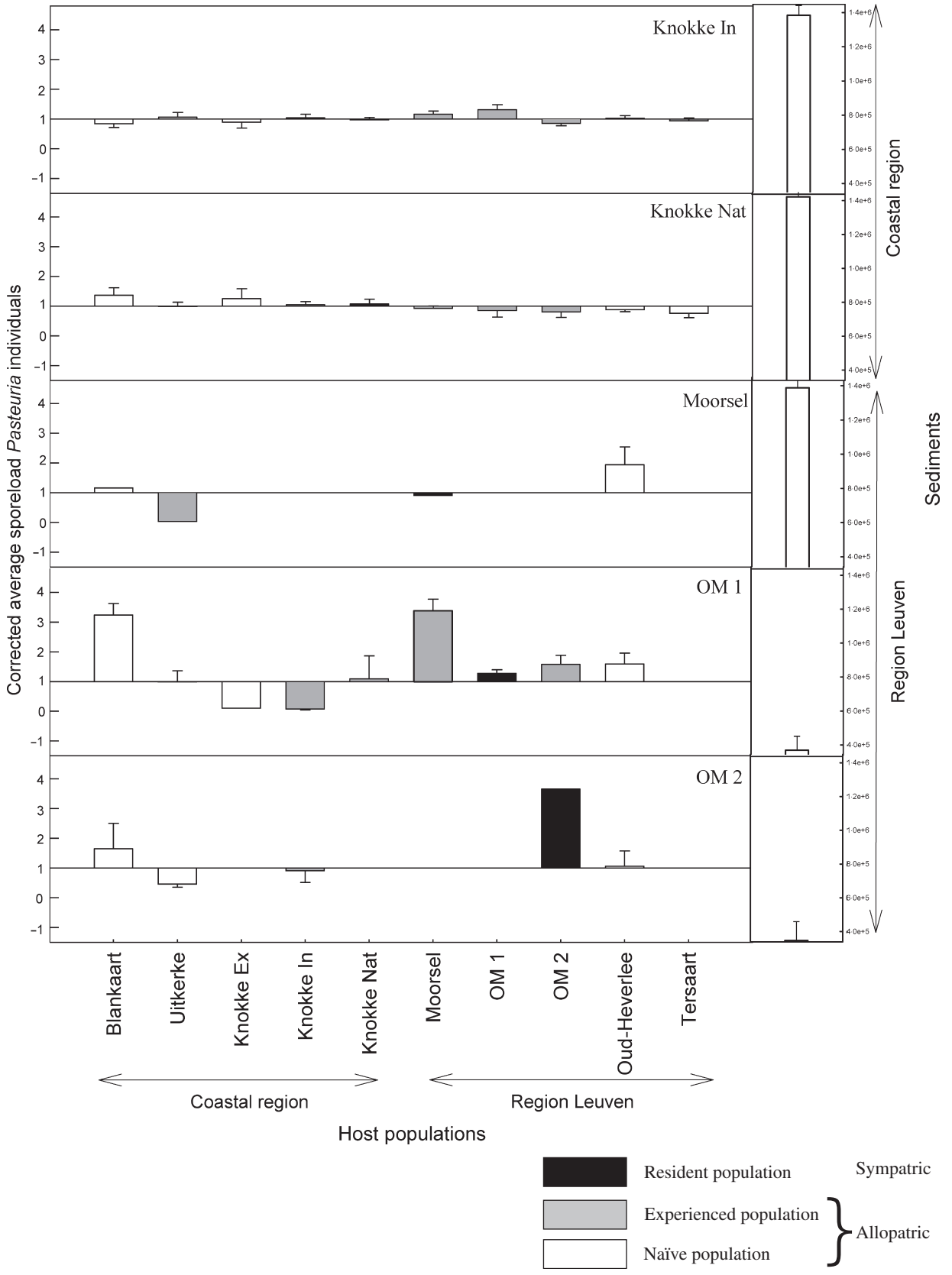

Fig. 5. Average (+1 SE) corrected spore load of *Pasteuria ramosa* in the 10 *Daphnia* host populations when exposed to the five sediments (Knokke In, Knokke Nat, Moorsel, OM 1 and OM 2) containing *P. ramosa* spores (separate panels). Spore loads were corrected by the average spore load of a given sediment (see Materials and methods and legend Fig. 2); these averages are given for each sediment in the right panel.

confronted with their local parasite population than when confronted with other parasite populations. Although for one epibiont, *Vorticella* sp., the inability to identify individuals to species level may have masked a pattern of local adaptation, our data suggest a different pattern for endoparasites and epibionts. The absence of any signal of local adaptation in epibionts, and more generally the absence of host population effects, probably reflects the absence of an intimate host–parasite interaction in this type of parasites (Stirnadel & Ebert 1997).

Our experiment not only reveals that sediment identity is important in determining parasite community composition, it also highlights that sediments of natural habitats differ strongly in the presence and relative abundance of microparasite infectious stages (as has been reported before; see Decaestecker *et al*. (2004)). Our results do not allow to identify why the studied parasite community in the different habitats is so different. Our multivariate analysis revealed that neither local environmental conditions nor geographical distances could explain a significant part of variation in parasite community structure. The density of *D. magna* dormant stages present in the pond sediment explained a minor part of the infection patterns, suggesting that host density to some extent contributed to the observed variation in parasite community composition. This effect, however, resulted mainly from differences in the microparasite community among the sediments and not from a correlation between infection intensities of individual microparasites and host dormant stage densities. Our results do not rule out an indirect effect of parasite or host local adaptation in influencing the abundance of parasite infectious stages in the sediments of the studied ponds. Yet, our data do not provide strong indications for such an effect.

We conclude that in our study local supply of parasite infectious stages is by far the most important factor determining parasite community composition in natural populations in *Daphnia*, while not excluding an impact of local genetic adaptation. Although local genetic adaptation and the underlying genotype by genotype interactions are no doubt important in determining host–parasite dynamics, they seem not to decisively impact parasite community composition on particular host populations.

Acknowledgement

We thank Sarah Rousseaux and Tom De Bie for providing data on the environmental characteristics of the ponds. M.J. enjoys a PhD fellowship of the Agency for Innovation by Science and Technology in Flanders (IWT, Flanders). A.C. benefited from a post-doctoral grant of the KULeuven Research Fund during this research. This research was financially supported by project GOA/08/06 of the KULeuven Research Fund and project G.0506.07 of FWO, Flanders.

References

Anderson, R.M. & May, R.M. (1986) The invasion, persistence and spread of infectious-diseases within animal and plant-communities. *Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences*, **314**, 533–570.

Carius, H.J., Little, T.J. & Ebert, D. (2001) Genetic variation in a host-parasite association: potential for coevolution and frequency-dependent selection. *Evolution*, **55**, 1136–1145.

Coors, A., Vanoverbeke, J., De Bie, T. & De Meester, L. (2009) Land use, genetic diversity and toxicant tolerance in natural populations of *Daphnia magna*. *Aquatic Toxicology*, **95**, 71–79.

Decaestecker, E., De Meester, L. & Ebert, D. (2002) In deep trouble: habitat selection constrained by multiple enemies in zooplankton. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 5481–5485.

Decaestecker, E., Lefever, C., De Meester, L. & Ebert, D. (2004) Haunted by the past: evidence for dormant stage banks of microparasites and epibionts of *Daphnia*. *Limnology & Oceanography*, **49**, 1355–1364.

Decaestecker, E., Gaba, S., Raeymaekers, J.A.M., Stoks, R., Van Kerckhoven, L., Ebert, D. & De Meester, L. (2007) Host-parasite 'Red Queen' dynamics archived in pond sediment. *Nature*, **450**, U816–U870.

DeStasio, B.T. (1993) Diel vertical and horizontal migration by zooplankton – population budgets and the diurnal deficit. *Bulletin of Marine Science*, **53**, 44–64.

Duncan, A.B. & Little, T.J. (2007) Parasite-driven genetic change in a natural population of *Daphnia*. *Evolution*, **61**, 796–803.

Duncan, A.B., Mitchel, l.S.E. & Little, T.J. (2006) Parasite-mediated selection and the role of sex and diapause in *Daphnia*. *Journal of Evolutionary Biology*, **19**, 1183–1189.

Ebert, D. (1994) Virulence and local adaptation of a horizontally transmitted parasite. *Science*, **265**, 1084–1086.

Ebert, D. (2005) *Ecology, Epidemiology and Evolution of Parasitism in Daphnia [Internet]*. National Library of Medicine (US), National Center for Biotechnology Information, Bethesda, MD. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db = Books, accessed 9/6/10.

Ebert, D. (2008) Host-parasite coevolution: insights from the *Daphnia*-parasite model system. *Microbiology*, **11**, 209–301.

Ebert, D., Zschokke-Rohringer, C.D. & Carius, H.J. (1998) Within- and between-population variation for resistence of *Daphnia magna* to the bacterial endoparasite *Pasteuria ramosa*. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, **265**, 2127–2134.

Ebert, D., Rainey, P., Embley, T.M. & Scholz, D. (1996) Development, life cycle, ultrastructure and phylogenetic position of *Pasteuria ramosa* Metchnikoff 1888: rediscovery of an obligate endoparasite of *Daphnia magna* Straus. *Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences*, **351**, 1689–1701.

Ebert, D., Carius, H.J., Little, T. & Decaestecker, E. (2004) The evolution of virulence when parasites cause host castration and gigantism. *The American Naturalist*, **164**(Suppl. 5), S19–S32.

Gaba, S. & Ebert, D. (2009) Time-shift experiments as a tool to study antagonistic coevolution. *Trends in Ecology and Evolution*, **24**, 226–232.

Gandon, S., Buckling, A., Decaestecker, E. & Day, T. (2008) Host-parasite coevolution and patterns of adaptation across time and space. *Journal of Evolutionary Biology*, **21**, 1861–1866.

Green, J. (1974) Parasites and epibionts of Cladocera. *The Zoological Society of London*, **32**, 417–515.

Haag, C.R. & Ebert, D. (2004) Parasite-mediated selection in experimental metapopulations of *Daphnia magna*. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, **271**, 2149–2155.

Havens, K.E. (1993) An experimental-analysis of macrozooplankton, microzooplankton and phytoplankton interactions in a temperate eutrophic lake. *Archiv fur Hydrobiologie*, **127**, 9–20.

Hill, M.O. & Gauch, H.G. (1980) Detrended correspondence analysis: an improved ordination technique. *Plant Ecology*, **42**, 47–58.

Kawecki, T.J. & Ebert, D. (2004) Conceptual issues in local adaptation. *Ecology Letters*, **7**, 1225–1241.

Klüttgen, B., Dülmer, U., Engels, M. & Ratte, H.T. (1994) ADaM, an artificial freshwater for the culture of zooplankton. *Water Research*, **28**, 743–746.

Lampert, W. (1987) Predictability in lake ecosystems: the role of biotic intercations. *Ecological Studies* (eds E.D. Schultze & H. Zwölfer), pp. 333–346. Springer-Verlag, Berlin/Heidelberg.

Larsson, J.I.R., Ebert, D., Mangin, K.L. & Vavra, J. (1998) Ultrastructural study and description of *Flabelliforma magnivora* sp n (*Microspora : Duboscqiidae*), a microsporidian parasite of *Daphnia magna* (Crustacea : Cladocera : *Daphniidae*). *Acta Protozoologica*, **37**, 41–52.

Legendre, P. & Legendre, L. (1998) *Numerical Ecology*. Elsevier, Science BV, Amsterdam.

Little, T.J., Watt, K. & Ebert, D. (2006) Parasite-host specificty: experimental studies on the basis of parasite adaptation. *Evolution*, **60**, 31–38.

Lively, C.M. (1989) Adaptation by a parasitic trematode to local-populations of its snail host. *Evolution*, **43**, 1663–1671.
Lively, C.M. (1999) Migration, virulence, and the geographic mosaic of adaptation by parasites. *The American Naturalist*, **153**, S34–S47.
Lively, C.M. & Dybdahl, M.F. (2000) Parasite adaptation to locally common host genotypes. *Nature*, **405**, 679–681.
Maccoll, A.D.C. (2009) Parasite burdens differ between sympatric three-spined stickleback species. *Ecography*, **32**, 153–160.
Mangin, K.L., Lipsitch, M. & Ebert, D. (1995) Virulence and transmission modes of two microsporidia in *Daphnia magna*. *Parasitology*, **111**, 133–142.
Manning, S.D., Woolhouse, M.E.J. & Ndamba, J. (1995) Geographic compatibility of the fresh-water snail bulinus-globosus and schistosomes from the Zimbabwe highveld. *International Journal for Parasitology*, **25**, 37–42.
Mopper, S. (1996) Adaptive genetic: structure in phytophagous insect populations. *Trends in Ecology and Evolution*, **11**, 235–238.
Refardt, D., Decaestecker, E., Johnson, P.T.J. & Vavra, J. (2008) Morphology, molecular phylogeny, and ecology of *Binucleata daphniae* n. g., n. sp (Fungi: *Microsporidia*), a parasite of *Daphnia magna* Straus, 1820 (Crustacea: *Branchiopoda*). *Journal of Eukaryotic Microbiology*, **55**, 393–408.
Stirnadel, H.A. & Ebert, D. (1997) Prevalence, host specificity and impact on host fecundity of microparasites and epibionts in three sympatric *Daphnia* species. *Journal of Animal Ecology*, **66**, 212–222.
Vogwill, T., Fenton, A. & Brockhurst, M.A. (2008) The impact of parasite dispersal on antagonistic host-parasite coevolution. *Journal of Evolutionary Biology*, **21**, 1252–1258.
Webster, J.P., Gower, C.M. & Blair, L. (2004) Do hosts and parasites coevolve? Empirical support from the Schistosoma system. *The American Naturalist*, **164** (Suppl.), S33–S51.

Received 6 December 2009; accepted 23 May 2010
Handling Editor: Rob Knell

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Fig. S1. Average (+1 SE) infection rate of *Amoebidium parasiticum*, in the 10 *Daphnia* host populations when exposed to the 10 sediments containing *A. parasiticum* spores (separate panels).
Fig. S2. Average (+1 SE) infection rate of *Vorticella* sp. in the 10 *Daphnia* host populations when exposed to the 10 sediments containing *Voricella* sp. spores (separate panels).
Fig. S3. Average (+1 SE) infection rate of *Brachionus rubens* in the 10 *Daphnia* host populations when exposed to the two sediments (different panels) containing *B. rubens* spores (Tersaart, Moorsel).

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials may be re-organized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.